



A new *Philautus* (Anura: Rhacophoridae) from northern Laos allied to *P. abditus* Inger, Orlov & Darevsky, 1999

BRYAN L. STUART^{1,4}, SOMPHOUTHONE PHIMMACHAK², SENGVILAY SEATEUN³ & JENNIFER A. SHERIDAN¹

¹North Carolina Museum of Natural Sciences, 11 West Jones Street, Raleigh NC 27601, USA

²Kasetsart University, Faculty of Science, Department of Zoology, Ngam Wong Wan Road, Chatuchak, Bangkok, 10900, Thailand

³National University of Laos, Faculty of Science, Department of Biology, P.O. Box 2273, Dong Dok Campus, Vientiane, Laos

⁴Corresponding author. E-mail: bryan.stuart@naturalsciences.org

Abstract

The small rhacophorid frog *Philautus abditus* is geographically restricted to central Vietnam and adjacent Cambodia. Our fieldwork in northern Laos resulted in the discovery of a *Philautus* species that very closely resembles *P. abditus*, but is at least 330 km from the nearest known locality of that species. The Laos population differs from *P. abditus* in mitochondrial DNA and coloration, and is described here as a new species. *Philautus nianeae* **sp. nov.** is distinguished from its congeners by having the combination of a hidden tympanum; no nuptial pads; smooth skin; large black spots on the hidden surfaces of the hind limbs; light venter with dark spotting; and a bronze iris. A second species of *Philautus* from northern Laos, *P. petilus*, is transferred on the basis of morphology to the genus *Theloderma*.

Key words: Laos; new species; *Philautus abditus*; *Philautus petilus*; Rhacophoridae

Introduction

The rhacophorid frog genus *Philautus* Gistel, 1848 has a turbulent taxonomic history (Bossuyt & Dubois 2001; Hertwig *et al.* 2012; Frost 2013). Eighty-four species of *Philautus* were recognized in a recent nomenclatural review (Bossuyt & Dubois 2001), but many of these species have since been transferred to other genera of rhacophorids, primarily on the basis of evidence derived from molecular phylogenetic analyses (e.g., Frost *et al.* 2006; Li *et al.* 2008; Li *et al.* 2009). No morphological synapomorphy is currently known for *Philautus*. The genus has been defined in the past as rhacophorids lacking vomerine teeth (Liem, 1970), or lacking a free-swimming aquatic larva (Bossuyt & Dubois 2001), but these definitions have not been supported by molecular phylogenetic analyses (Hertwig *et al.* 2012). As currently recognized, the genus *Philautus* contains approximately 50 species (Frost 2013), most of which are relatively small and arboreal. The genus is distributed from India to the Philippines, with the greatest species diversity occurring in Borneo and the Philippines (Brown & Alcala 1994; Hertwig *et al.* 2012; Frost 2013).

Our recent fieldwork in northern Laos revealed a *Philautus* that bears remarkable similarity to *P. abditus* Inger, Orlov & Darevsky 1999, a species that is restricted to central Vietnam and adjacent northeastern Cambodia (Nguyen *et al.* 2009; Stuart *et al.* 2010) at least 330 km southeast of the Laos locality (the nearest known record of *P. abditus* is in Quang Nam Province, Vietnam; Figure 1). Despite the similarity, the Laos specimens differ from *P. abditus* in coloration and mitochondrial DNA (the call of *P. abditus* is unknown), and are herein described as a new species.

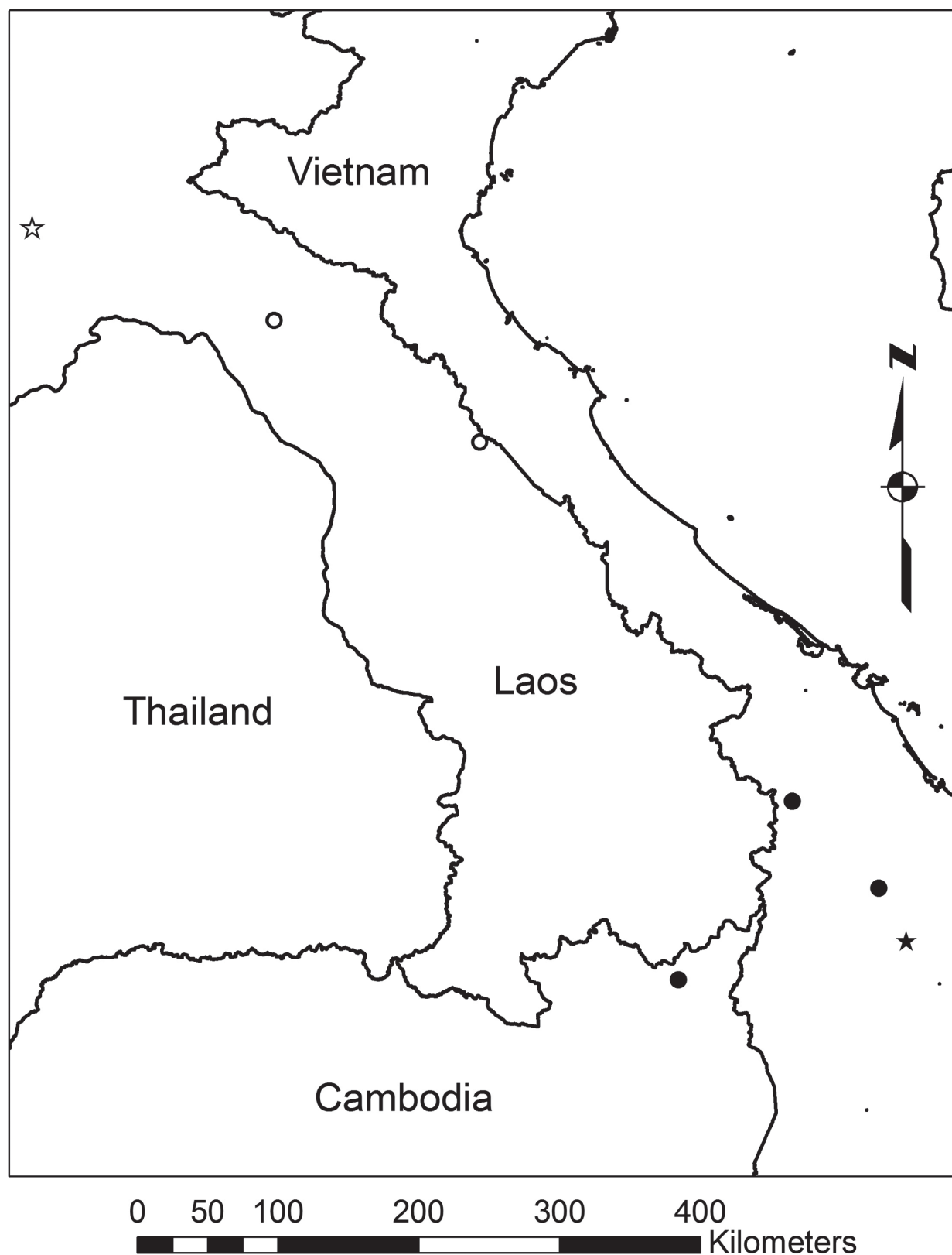


FIGURE 1. Map illustrating the type locality (white star) of *Philautus nianeae* **sp. nov.** in Xaysomboun District, Vientiane Province, Laos; the paratype localities (white circles) of *P. nianeae* **sp. nov.** in Viengthong District, Bolikhamxay Province, Laos and Boualapha District, Khammouan Province, Laos; the type locality (black star) of *P. abditus* in An Khe District, Gia Lai Province, Vietnam (Inger *et al.* 1999); and all other reported localities (black circles) of *P. abditus* in Kon Plong District, Kon Tum Province, Vietnam (Nguyen *et al.* 2009), Phuoc Son District, Quang Nam Province, Vietnam (this study), and Veunsai District, Ratanakiri Province, Cambodia (Stuart *et al.* 2010).

Materials and methods

Sampling. Specimens were collected by hand and fixed in 10% buffered formalin after preserving liver in 20% DMSO-salt saturated storage buffer and RNAlater (Ambion). Specimens were later transferred to 70% ethanol. Specimens and tissue samples were deposited at the North Carolina Museum of Natural Sciences (NCSM) and the National University of Laos, Faculty of Science, Department of Biology (NUOL).

Morphology. Specimens of *P. abditus* were examined for comparison in the holdings of NCSM, Field Museum of Natural History (FMNH), Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), and Australian Museum (AMS): FMNH 252833 (holotype male), FMNH 252834, MVZ 222118, MVZ 222121 (paratype males), MVZ 222119–20 (paratype females), FMNH 252836, FMNH 252838 (paratype juveniles), Vietnam, Gia Lai Province, An Khe District, Buon Loi; AMS R 171540 (male), AMS R 171541, NCSM 79188 (females), Vietnam, Quang Nam Province, Phuoc Son District, Song Thanh Nature Reserve; MVZ 258310 (juvenile), Cambodia, Ratanakiri Province, Veunsai District, Virachey National Park.

Measurements were taken to the nearest 0.1 mm with dial calipers: snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); maximum head width (HDW); snout length from tip of snout to anterior corner of eye (SNT); eye diameter (EYE); interorbital distance (IOD); internasal distance (IND); shank length (SHK); thigh length (TGH); forearm length, from tip of third digit to elbow (FAL); manus length from tip of third digit to base of outer palmar tubercle (HND); horizontal diameter of disk on third finger (F3D); pes length from tip of fourth toe to base of inner metatarsal tubercle (FTL); and horizontal diameter of disk on fourth toe (T4D).

Molecules. Total genomic DNA was extracted from liver using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A 1,032–1,035 nucleotide basepair (bp) fragment of mitochondrial DNA that encodes part of the 16S rRNA gene was amplified by PCR (the polymerase chain reaction; one cycle of 94°C 5 min, 35 cycles of 94°C 45 s, 60°C 30 s, 72°C 1 min, one cycle of 72°C 10 min) using the primer pairs L-16SRana (5'-CCTACCGAGCTTAGAGATAGC-3') / H-16SRanaIII (Stuart *et al.* 2006), and 16Sar-5' / 16Sbr-3' (Palumbi 1996). PCR products were cleaned using ExoSAP-IT (USB). Cycle sequencing products were sequenced in both directions on a 3130 DNA Analyzer (Applied Biosystems) using the amplifying primers and Big Dye version 3 chemistry (Perkin Elmer). Sequences were edited with Sequencher v. 4.1 (Genecodes) and deposited in GenBank under accession numbers JX885770–JX885772, KF723225–KF723234. A homologous sequence from a near topotype of *P. abditus* from Krong Pa District, Gia Lai Province, Vietnam was downloaded from GenBank and included in the analysis [GenBank accession GQ285673 of voucher Royal Ontario Museum (ROM) 33145]. Pairwise distances were calculated using PAUP* 4.0b10 (Swofford 2002).

Calls. Advertisement calls were recorded with an Edirol R-09HR WAVE/MP3 Recorder (96 kHz sampling rate and 24-bit encoding) at a distance of approximately 0.5 m. Ambient weather conditions were taken immediately after the recording using a Kestrel 3500 hand-held weather meter. Calls were analyzed with Raven Pro 1.4 (Bioacoustics Research Program 2011). Call parameters represent the mean of six calls recorded within approximately five minutes. Call duration (s), inter-call interval (s), dominant frequency (Hz), number of pulses, and pulse rate (pulses/s) were measured. Audiospectrograms were created with fast-Fourier transform (FFT) of 512 points, 50% overlap.

Results

Philautus nianeae sp. nov.

Holotype. NCSM 80038 (field tag BLS 15379), adult male (Figure 2), Laos, Vientiane Province, Xaysomboun District, Nam Ngum River, 19.01807°N 102.87633°E, 490 m elev., coll. 10 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay.

Paratypes. Seven adult males: NUOL 00004, NCSM 80039–41, same locality as holotype except 19.01622°N 102.87688°E, 493 m elev., coll. 11 May 2012. NCSM 80042, same data as holotype except Houay Men Stream, tributary of Nam Pha River, 19.04507°N 102.89150°E, 548 m elev., coll. 14 May 2012. NCSM 80043, same data as holotype except Houay Men Stream, tributary of Nam Pha River, 19.04620°N 102.89136°E, 530 m elev., coll. 14 May 2012. NCSM 80044, same data as holotype except Houay Men Stream, tributary of Nam Pha River, 19.04532°N 102.89143°E, 553 m elev., coll. 15 May 2012.

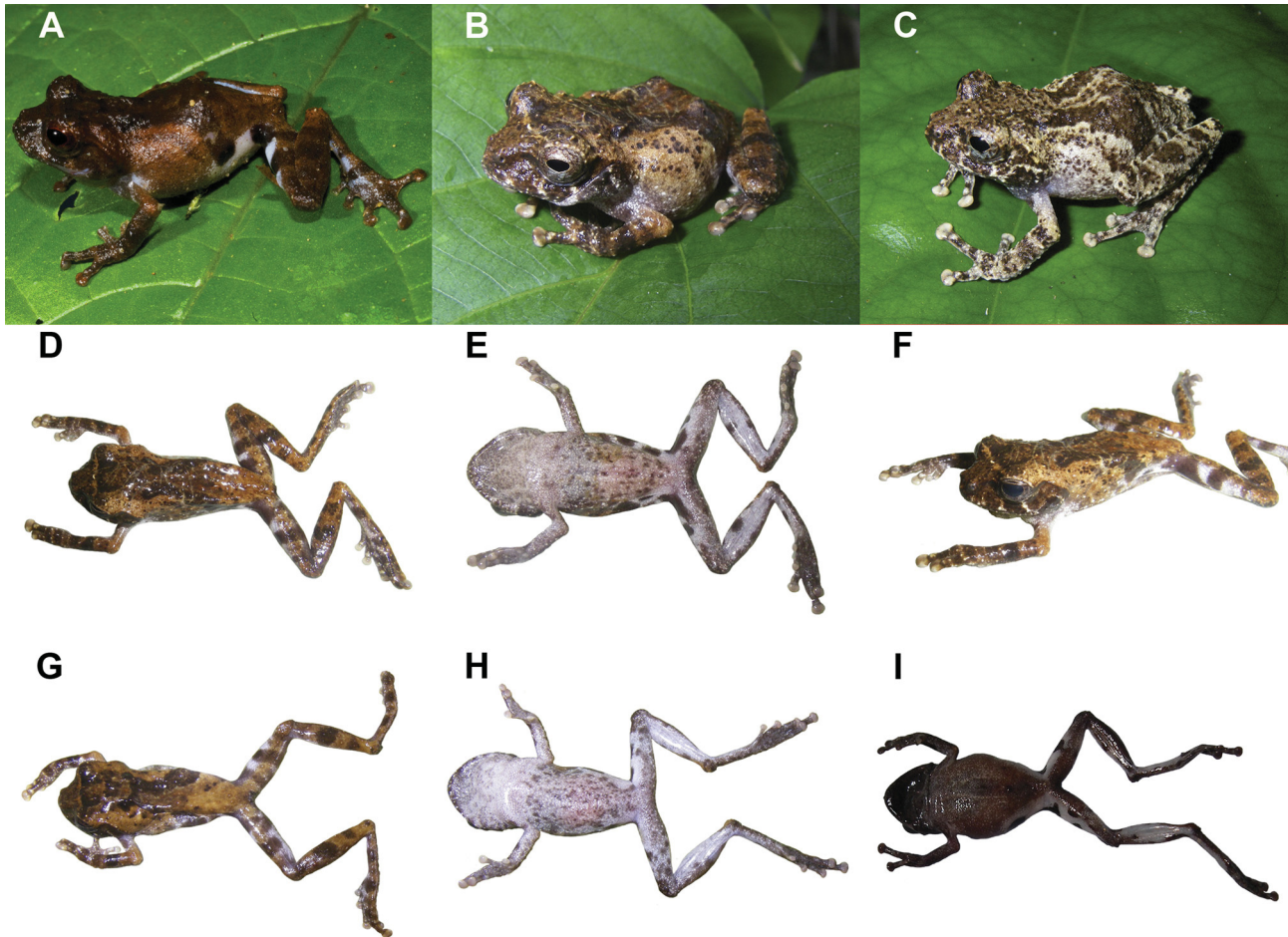


FIGURE 2. (A) *Philautus abditus* in life from Quang Nam Province, Vietnam. Photograph by J. J. L. Rowley. (B) Holotype NCSM 80038 of *P. nianeae* **sp. nov.** in life. Photograph by B. L. Stuart. (C) Paratype NCSM 80926 of *P. nianeae* **sp. nov.** in life. Photograph by B. L. Stuart. (D) Dorsal, (E) ventral, and (F) lateral views of holotype NCSM 80038 of *P. nianeae* **sp. nov.** immediately prior to preservation. (G) Dorsal and (H) ventral views of paratype NCSM 80042 of *P. nianeae* **sp. nov.** immediately prior to preservation. (I) Ventral view of *P. abditus* from Quang Nam Province, Vietnam immediately prior to preservation. Photograph by J. J. L. Rowley.

One adult female: NCSM 80690, Laos, Bolikhamxay Province, Viengthong District, Nam Kading National Protected Area, 18.42195°N, 104.42139°E, 471 m elev., coll. 3 March 2013 by Bryan L. Stuart, Niane Sivongxay, Sengvilay Seateun, and Jennifer A. Sheridan. Three juveniles: NCSM 80691, NUOL 00011, same data as NCSM 80690. NCSM 80689, same data as NCSM 80690 except 18.42290°N 104.42210°E, 488 m elev., coll. 2 March 2013.

Three adult males: NCSM 80926, Laos, Khammouan Province, Boualapha District, Nakai-Nam Theun National Protected Area, Phou Ack Mountain, 17.64466°N 105.73521°E, 972 m elev., coll. 12 May 2013 by Bryan L. Stuart, Somphouthone Phimmachak, and Jennifer A. Sheridan. NCSM 80927, same data as NCSM 80926 except 17.64425°N 105.73526°E, 979 m elev., coll. 13 May 2013. NUOL 00012, same data as NCSM 80926 except 17.64516°N 105.73626°E, 974 m elev., coll. 14 May 2013.

Etymology. The specific epithet is a matronym for Dr. Niane Sivongxay, Professor of Biology at the National University of Laos, co-collector of the species, and cherished friend and colleague of the authors.

Diagnosis. The new species is assigned to *Philautus* on the basis of its very close morphological similarity, and likely sister taxon relationship, to *P. abditus*, a species that is phylogenetically nested within a clade containing the generotype *P. aurifasciatus* (Schlegel 1837) (see Li *et al.* 2009; Pyron & Wiens 2011; Hertwig *et al.* 2012). The new species is a medium-sized *Philautus* having males with SVL 23.8–28.4, female with SVL 27.4; smooth skin, without spines or tubercles; tympanum completely hidden under skin; no nuptial pads; no dermal fringes or tubercles on limbs; extensive webbing on the foot; bronze iris; light-colored dorsal surfaces of discs on fingers and

toes; ventral surfaces light gray with dark spotting; and large black spots on light background in inguinal region and hidden surfaces of hind limb.

Description of holotype. Habitus stocky. Head length subequal to head width. Snout rounded in dorsal and lateral views, a feeble prominence at tip; nostril as vertical slit, much closer to tip of snout than to eye, internarial shorter than interorbital distance; canthus rostralis distinct, rounded, constricted behind nares; lores oblique, concave; eye diameter greater than snout length, interorbital distance greater than upper eyelid width; tympanum not visible, hidden under skin; dentigerous process of vomer and vomerine teeth absent; choanae oval, separated by a distance equal to approximately four times horizontal diameter of choanae; tongue heart-shaped, deeply notched posteriorly.

Forelimb slender. Finger tips with round discs having circummarginal grooves; fingers moderately slender; relative finger lengths $I < II < IV < III$; outer three fingers with rudiment of web at base; subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2; two oval, outer palmar tubercles in contact, lateral largest, surfaces flat; oval, thenar tubercle, surface flat; round, indistinct supernumerary tubercles.

Hindlimb slender. Toe tips with round discs having circummarginal grooves, diameter of discs subequal to those of fingers; toes moderately slender; relative toe lengths $I < II < III < V < IV$; web on toe I and preaxial side of toe II to level of distal margin of subarticular tubercle, on postaxial side of toe II to base of tip, on preaxial side of toe III to level of proximal subarticular tubercle, on postaxial side of toe III to base of tip, on preaxial and postaxial sides of toe IV to level of penultimate subarticular tubercle and continuing as a fringe to base of tip, and on toe V to level of distal margin of subarticular tubercle; weak dermal fringe on outer margin of toe V from base of foot to base of tip; subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 3, 2; inner metatarsal tubercle oval, surface flat; outer metatarsal tubercle absent.

Skin smooth dorsally and laterally; weak tubercles on dorsal surface of head and near vent; curved supratympanic fold; venter finely granular; no dermal fringes, flaps, or tubercles on limbs.

Nuptial pads absent; elongated vocal sac openings near corner of mouth; vocal sac median; testes mature.

Color of holotype in life. Iris bronze; white line extending from under eye to corner of mouth, with irregular white bars on upper lip; dorsum, flank, and dorsal surfaces of forelimbs, thigh and shank brown; indistinct black X-shaped marking on back extending to near groin; irregular black cross bar between eyelids; broad black crossbands on dorsal surfaces of limbs; axillary region white; inguinal region, anterior surface and poster surfaces of thigh, ventral surfaces of shank, and dorsal surface of foot white or light gray with large black spots, often formed by terminus of black crossband on limb; dorsal surfaces of discs on fingers and toes white and brown; throat, belly, and ventral surfaces of forelimb and thigh light gray with dark gray, brown and black spotting.

Color of holotype in preservative. Gray on throat and belly faded to light brown.

Variation. Paratypes closely resemble the holotype, varying mostly in the presence and number of large black spots on the ventral surface of the shank. The holotype, NCSM 80039, NCSM 80690, and NUOL 00004 have one large black spot on each shank, NCSM 80926 has one spot on the right shank but none on the left, NCSM 80040 and NCSM 80043 have two spots on the right shank but none on the left, NCSM 80041 and NCSM 80927 have two spots on each shank, NUOL 00012 has three spots on each shank, and NCSM 80042 and NCSM 80044 have none on either shank. A single female (NCSM 80690) with developing, pigmented ova is similar in size to mature males (Table 1). Measurements are summarized in Table 1.

Molecules. Ten paratypes of *P. nianeae* sampled among the three Laos localities (NCSM 80039, NCSM 80043–44, NCSM 80689–91, NCSM 80926–27, NUOL 00011–12) have uncorrected pairwise distances of 0.00–0.97% in the 16S gene fragment, but have uncorrected pairwise distances of 2.77–3.26% in the same gene fragment to a near topotype of *P. abditus* from Gia Lai Province, Vietnam (ROM 33145), 3.49–3.78% to two specimens of *P. abditus* from Quang Nam Province, Vietnam (AMS R 171540–41) and 2.52–2.61% from a specimen of *P. abditus* from Ratanakiri Province, Cambodia (MVZ 258310; Table 2).

Advertisement call. Call description is based on a single recording of paratype NCSM 80926, recorded at 22.8° C ambient temperature, 88.8% relative humidity, and 902.0 hPa atmospheric pressure at 2000 h. The recording contained six calls with an inter-call interval of mean \pm SD 49.38 ± 5.23 s. Calls consisted of two notes (Figure 3). The first note had 1–3 (2 ± 0.89) pulses and the second note had 10–15 (12 ± 1.67) pulses. Pulse rate for the second note was 4.57 ± 0.07 pulses per second. Call duration was 4.15 ± 0.42 s, with dominant frequency of 2.6 ± 0.0 kHz for the first note, and 2.5 ± 0.1 kHz for the second note.

TABLE 1. Measurements (mm) of adult *Philautus nianae* sp. nov and *P. abditus*. Abbreviations defined in the text.

Measurement	<i>P. nianae</i> sp. nov. Holotype Male NCSM 80038	<i>P. nianae</i> sp. nov. All Males <i>n</i> = 11	<i>P. nianae</i> sp. nov. Paratype Female NCSM 80690	<i>P. abditus</i> Males <i>n</i> = 5	<i>P. abditus</i> Females <i>n</i> = 4
		Range; Mean \pm SD		Range; Mean \pm SD	Range; Mean \pm SD
SVL	24.9	23.8–28.4; 25.7 \pm 1.5	27.4	25.4–28.4; 26.7 \pm 1.2	24.6–28.7; 27.2 \pm 1.8
HDL	10.1	9.1–10.9; 10.1 \pm 0.7	11.0	9.8–10.4; 10.2 \pm 0.3	10.2–11.2; 10.8 \pm 0.4
HDW	10.0	9.4–10.9; 10.1 \pm 0.5	10.8	10.2–10.8; 10.6 \pm 0.3	10.5–11.5; 11.0 \pm 0.5
SNT	3.7	3.5–4.5; 4.0 \pm 0.4	4.3	3.9–4.3; 4.0 \pm 0.2	3.9–4.2; 4.1 \pm 0.1
EYE	4.0	3.6–4.3; 3.9 \pm 0.2	4.9	4.2–4.6; 4.4 \pm 0.2	4.3–4.8; 4.6 \pm 0.2
IOD	3.1	3.0–3.5; 3.2 \pm 0.2	3.1	3.2–3.6; 3.3 \pm 0.2	3.0–4.0; 3.6 \pm 0.4
IND	2.6	2.6–3.0; 2.8 \pm 0.2	2.8	2.7–3.0; 2.8 \pm 0.1	2.6–3.0; 2.8 \pm 0.2
SHK	12.7	11.6–13.8; 12.8 \pm 0.7	13.4	12.4–13.3; 12.8 \pm 0.3	13.0–14.2; 13.7 \pm 0.6
TGH	11.9	11.6–14.2; 12.7 \pm 0.9	13.7	11.9–13.0; 12.5 \pm 0.4	12.7–13.4; 13.1 \pm 0.3
FAL	12.3	11.3–14.1; 12.7 \pm 0.8	13.5	12.4–13.0; 12.7 \pm 0.2	12.7–14.6; 13.9 \pm 0.8
HND	6.9	6.0–7.9; 7.0 \pm 0.5	7.7	7.2–7.7; 7.4 \pm 0.2	7.4–8.4; 7.8 \pm 0.5
F3D	1.3	1.0–1.4; 1.2 \pm 0.1	1.2	1.4–1.8; 1.6 \pm 0.2	1.2–1.9; 1.6 \pm 0.3
FTL	10.0	9.3–11.1; 10.2 \pm 0.6	11.0	9.9–10.7; 10.3 \pm 0.3	10.3–11.4; 10.7 \pm 0.5
T4D	1.4	1.0–1.5; 1.3 \pm 0.2	1.2	1.3–1.7; 1.5 \pm 0.2	1.1–1.8; 1.4 \pm 0.3

TABLE 2. Uncorrected pairwise distances (%) of the 16S rRNA gene from *Philautus nianeae* sp. nov. and *P. abditus*.

Species	<i>P. nianeae</i> sp. nov.	<i>P. nianeae</i> sp. nov.	<i>P. nianeae</i> sp. nov.	<i>P. nianeae</i> sp. nov.	<i>P. abditus</i>	<i>P. abditus</i>	<i>P. abditus</i>
Locality	Vientiane Prov.	Bolikhamxay Prov.	Khammouan Prov.	Gia Lai Prov.	Quang Nam Prov.	Ratanakiri Prov.	
Voucher							
GenBank Accession							
<i>P. nianeae</i> sp. nov.	0.00–0.10						
Vientiane Prov.							
NCSM 80039, 80043–44							
JX885770–72							
<i>P. nianeae</i> sp. nov.	0.87–0.97	0.00					
Bolikhamxay Prov.							
NCSM 80689–91, NUOL 00011							
KF723228–KF723231							
<i>P. nianeae</i> sp. nov.	0.87–0.97	0.19	0.00				
Khammouan Prov.							
NCSM 80926–27, NUOL 00012							
KF723232–KF723234							
<i>P. abditus</i>	2.77–2.87	3.26	3.26	–			
Gia Lai Prov.							
ROM 33145							
GQ285673							
<i>P. abditus</i>	3.49–3.59	3.78	3.78	2.78	0.00		
Quang Nam Prov.							
AMS R 171540–41							
KF723225–KF723226							
<i>P. abditus</i>	2.52–2.61	2.61	2.61	2.67	2.52	–	
Ratanakiri Prov.							
MVZ 258310							
KF723227							

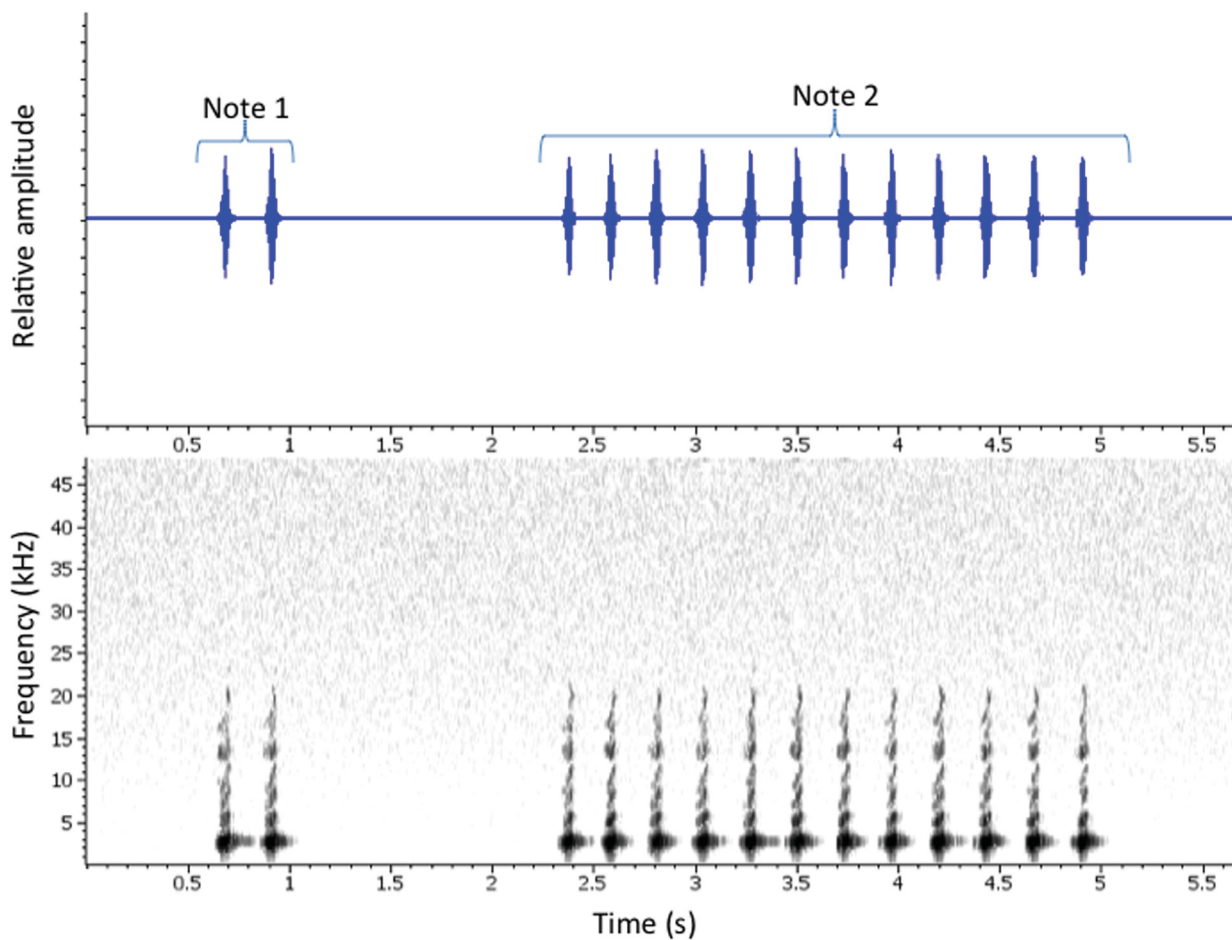


FIGURE 3. (Above) Waveform and (below) spectrogram of an advertisement call of paratype NCSM 80926 of *Philautus nianeae* sp. nov. obtained during a 5-minute recording at an ambient air temperature of 22.8° C.

Distribution and natural history. *Philautus nianeae* is known from three localities in Vientiane, Bolikhamxay, and Khammouan Provinces in northern Laos (Figure 1). In Vientiane Province, males were found during mid-May calling at night (2015–2130 h) on vegetation within 2 m of the ground and within 5 m of streams and riverbanks in disturbed semi-evergreen forest, sometimes mixed with bamboo, at 490–548 m elevation. In Bolikhamxay Province, a female and three juveniles were found during early March on rainy nights (1912–1952 h) 1 m above the ground on sapling leaves and palm fronds within 3 m of small rocky streams in semi-evergreen forest at 471–488 m elevation. In Khammouan Province, males were found during mid-May calling at night (2000–2110 h) on vegetation 1.5–4 m above the ground in semi-evergreen mixed with pine forest near the edge of open grassland at 972–979 m elevation. The Nam Ngum River at the type locality in Vientiane Province is under concession for a hydroelectric power project, making the persistence of the species at the type locality uncertain.

Comparisons. The combination of having a hidden tympanum and large black spots on hidden surfaces of the hind limbs distinguishes *P. nianeae* from all other species of *Philautus* except *P. abditus*. *Philautus nianeae* differs from *P. abditus* by having a bronze iris (*P. abditus* with red iris); the throat, belly, and ventral surfaces of forelimb and thigh light gray with dark spotting (*P. abditus* with throat, belly, and ventral surfaces of forelimb and thigh dark gray or black); considerably less contrast between large black spots and light background in inguinal region and hidden surfaces of hindlimbs (*P. abditus* having strongly contrasting large black spots with sharp borders on an immaculate white background in inguinal region and hidden surfaces of hindlimbs); and light-colored dorsal surfaces of discs on fingers and toes (*P. abditus* with dark-colored dorsal surfaces of discs on fingers and toes).

Two additional species of *Philautus* occur in the vicinity of Laos, *P. maosonensis* Bourret 1937 from northern Vietnam and *P. cardamonus* Ohler, Swan & Daltry 2002 from southwestern Cambodia. *Philautus nianeae* further differs from these two species by having smooth skin (*P. maosonensis* males with distinct conical tubercles on head; Orlov *et al.* 2004), lacking yellow fingertips (present in *P. maosonensis*; Bain & Nguyen 2004), lacking a

greenish-yellow belly and ventral surface of limbs (present in *P. cardamonus*; Ohler *et al.* 2002), and lacking nuptial pads (present in *P. cardamonus*; Ohler *et al.* 2002). *Theloderma petilum* (Stuart & Heatwole 2004) **new comb.**, known only by its female holotype from northern Laos, was provisionally placed in *Philautus* in its original description (as *P. petilus*). However, this species is almost certainly related to a suite of species also having a slender habitus, dorsal dermal asperities, distinct tympanum, and no finger webbing, including *T. nebulosum* Rowley, Le, Hoang, Dau & Cao 2011, *T. palliatum* Rowley, Le, Hoang, Dau & Cao 2011, *T. rhododiscus* (Liu & Hu, 1962), and *T. truongsongense* (Orlov & Ho 2005), that have been recently placed (either as newly described species or transferred from *Philautus*) into an expanded *Theloderma* Tschudi 1838 on the basis of molecular phylogenetic analyses (Yu *et al.* 2008; Rowley *et al.* 2011).

Discussion

The discovery of *P. nianeae* **sp. nov.** adds an additional species of true *Philautus* to Indochina, a genus that is otherwise primarily found in Borneo and the Philippines, following reassessment of its species content based on molecular phylogenetic analyses (e.g., Frost *et al.* 2006; Li *et al.* 2008; Li *et al.* 2009; Hertwig *et al.* 2012).

The differences between *P. nianeae* **sp. nov.** and *P. abditus* are not large, but are comparable to those observed between other closely related anuran species. Many sister species of Madagascar frogs also exhibit approximately 3% divergence in the 16S rRNA gene (Vieites *et al.* 2009). Eye coloration is considered a taxonomically important character in frogs (Glaw & Vences 1997; Stuart *et al.* 2011), and at least two species of Southeast Asian rhacophorid frogs are diagnosed primarily on the basis of iris coloration (Stuebing & Wong 2000; Das 2005). Unfortunately, the advertisement call of *P. abditus* remains undescribed, but the description provided here of the call of *P. nianeae* **sp. nov.** should allow for that comparison in the future. *Philautus abditus* is endemic to the Central Highlands (or Kon Tum Plateau) of Vietnam and adjacent parts of Cambodia and probably Laos, an area with high faunal endemism (Sterling *et al.* 2006), inferring that a biogeographic barrier probably exists between the geographic ranges of *P. abditus* and *P. nianeae* **sp. nov.**

Considerable genetic variation was also found within *P. abditus*, as the species is currently recognized. One specimen of *P. abditus* from Gia Lai Province, Vietnam, two from Quang Nam Province, Vietnam, and one from Ratanakiri Province, Cambodia, have a maximum uncorrected pairwise distance of 2.78% in the 16S rRNA gene fragment (Table 2). No morphological differences are apparent in the preserved specimens from Gia Lai and Quang Nam Provinces. However, the bright yellow hindlimb coloration observed in life in the juvenile from Cambodia (Figure 4 in Stuart *et al.* 2010) was not found in adults from Vietnam (Figure 2; Orlov *et al.* 2004; Nguyen *et al.* 2009; J. J. L. Rowley and D. A. Kizirian, unpublished data). Whether this hindlimb coloration represents ontogenetic or geographic variation remains unknown. Additional species diversity may be hidden within *P. abditus*.

Acknowledgements

Fieldwork in Vientiane Province was conducted under the auspices of the Nam Ngum 3 Power Company. Fieldwork in Bolikhamxay and Khammouan Provinces was conducted as part of the Biodiversity Conservation Project, a cooperative program between the National University of Laos and the Wildlife Conservation Society Laos Program. François Obein, Sompak Sattayasoonorn, Troy Hansel, and Alex McWilliam provided critical logistical support. Niane Sivongxay assisted with fieldwork. The Ministry of Natural Resources and Environment, Nam Kading and Nakai-Nam Theun National Protected Area staff, and the Nam Theun 2 Watershed Management and Protection Authority granted permission for fieldwork. The Ministry of Natural Resources and Environment, Department of Forest Resource Management, CITES Management Authority, Vientiane, provided specimen export permits to the North Carolina Museum of Natural Sciences. Alan Resetar (FMNH), Jim McGuire and Carol Spencer (MVZ), and Ross Sadler and Jodi Rowley (AMS) loaned specimens in their care. Jodi Rowley provided photographs of *P. abditus*, Jonathan Raine constructed the map, and Peter Narins assisted with the call analysis. Jodi Rowley, Rafe Brown, and an anonymous reviewer improved the manuscript. The Nam Ngum 3 Power Company and the National Science Foundation (DEB-1145922) supported this work.

References

- Bain, R.H. & Nguyen, T.Q. (2004) Herpetofaunal diversity of Ha Giang Province in northeastern Vietnam, with descriptions of two new species. *American Museum Novitates*, 3453, 1–42.
[http://dx.doi.org/10.1206/0003-0082\(2004\)453<0001:hdohgp>2.0.co;2](http://dx.doi.org/10.1206/0003-0082(2004)453<0001:hdohgp>2.0.co;2)
- Bioacoustics Research Program (2011) Raven Pro: Interactive Sound Analysis Software (Version 1.4) The Cornell Lab of Ornithology, Ithaca, New York. Available from: <http://www.birds.cornell.edu/raven> (accessed 12 November 2013)
- Bossuyt, F. & Dubois, A. (2001) A review of the frog genus *Philautus* Gistel, 1848 (Amphibia, Anura, Ranidae, Rhacophorinae). *Zeylanica*, 6, 1–112.
- Brown W.C. & Alcalá, A.C. (1994) Philippine frogs of the family Rhacophoridae. *Proceedings of the California Academy of Sciences*, 48, 185–220.
- Bourret, R. (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences naturelles de l'Université. Descriptions de quinze espèces ou variétés nouvelles. *Annexe au Bulletin Général de l'Instruction Publique*, 4, 5–56.
- Das, I. (2005) A new species of *Polypedates* (Anura: Rhacophoridae) from Gunung Murud, Sarawak (northwestern Borneo). *The Raffles Bulletin of Zoology*, 53, 265–270.
- Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P.E., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler, W.C. (2006) The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297, 1–370.
[http://dx.doi.org/10.1206/0003-0090\(2006\)297\[0001:tatol\]2.0.co;2](http://dx.doi.org/10.1206/0003-0090(2006)297[0001:tatol]2.0.co;2)
- Frost, D.R. (2013) Amphibian Species of the World: an Online Reference. Version 5.6 (9 January 2013). American Museum of Natural History, New York. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 1 June 2013)
- Gistel, J.v.N.F.X. (1848) *Naturgeschichte des Thierreichs. Für höhere Schulen*. Hoffmann, Stuttgart, 216 pp.
- Glaw, F. & Vences, M. (1997) Anuran eye colouration: definitions, variation, taxonomic implications and possible functions. In: Böhme, W., Bischoff, W. & Ziegler, T. (Eds.), *Herpetologia Bonnensis. Proceedings of the 8th Ordinary General Meeting of the Societas Europaea Herpetologica, 23–27 August 1995*. Societas Europaea Herpetologica, Deutsche Gesellschaft für Herpetologie und Terrarienkunde and Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, pp. 125–138.
- Hertwig, S.T., Das, I., Schweizer, M., Brown, R. & Haas, A. (2012) Phylogenetic relationships of the *Rhacophorus everetti*-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta*, 41, 29–46.
<http://dx.doi.org/10.1111/j.1463-6409.2011.00499.x>
- Inger, R.F., Orlov, N. & Darevsky, I. (1999) Frogs of Vietnam: a report on new collections. *Fieldiana, New Series*, 92, 1–46.
<http://dx.doi.org/10.5962/bhl.title.3478>
- Li, J., Che, J., Bain, R.H., Zhao, E. & Zhang, Y. (2008) Molecular phylogeny of Rhacophoridae (Anura): a framework of taxonomic reassignment of species within the genera *Aquixalus*, *Chiromantis*, *Rhacophorus*, and *Philautus*. *Molecular Phylogenetics and Evolution*, 48, 302–312.
<http://dx.doi.org/10.1016/j.ympev.2008.03.023>
- Li, J., Che, J., Murphy, R.W., Zhao, H., Zhao, E., Rao, D. & Zhang, Y. (2009) New insights to the molecular phylogenetics and generic assessment in the Rhacophoridae (Amphibia: Anura) based on five nuclear genes and three mitochondrial genes, with comments on the evolution of reproduction. *Molecular Phylogenetics and Evolution*, 53, 509–522.
<http://dx.doi.org/10.1016/j.ympev.2009.06.023>
- Liem, S.S. (1970) The morphology, systematics, and evolution of the Old World treefrogs (Rhacophoridae and Hyperoliidae). *Fieldiana Zoology*, 57, 1–145.
- Liu, C.-C. & Hu, S.-Q. (1962) A herpetological report of Kwangsi. *Acta Zoologica Sinica*, 14 (Suppl.), 73–104.
- Nguyen, V.S., Ho, C.T. & Nguyen, T.Q. (2009) *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt am Main, 768 pp.
- Ohler, A., Swan, S.R. & Daltry, J.C. (2002) A recent survey of the amphibian fauna of the Cardamom Mountains, Southwest Cambodia with descriptions of three new species. *The Raffles Bulletin of Zoology*, 50, 465–481.
- Orlov, N.L. & Ho, C.T. (2005) A new species of *Philautus* from Vietnam (Anura: Rhacophoridae). *Russian Journal of Herpetology*, 12, 135–142.
<http://dx.doi.org/10.5358/hjsj.22.51>
- Orlov, N.L., Ho, C.T. & Nguyen, T.Q. (2004) A new species of the genus *Philautus* from Central Vietnam (Anura: Rhacophoridae). *Russian Journal of Herpetology*, 11, 51–64.
<http://dx.doi.org/10.5358/hjsj.22.51>
- Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematics*. Second edition. Sinauer Associates, Inc., Sunderland, Massachusetts, pp. 205–247.
- Pyron, A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61, 543–583.
<http://dx.doi.org/10.1016/j.ympev.2011.06.012>

- Rowley, J.J.L., Le, D.T.T., Hoang, H.D., Dau, V.Q. & Cao, T.T. (2011) Two new species of *Theloderma* (Anura: Rhacophoridae) from Vietnam. *Zootaxa*, 3098, 1–20.
- Schlegel, H. (1837) *Abbildungen neuer oder unvollständig bekannter Amphibien, nach der Natur oder dem Leben entworfen, herausgegeben und mit einem erläuternden Texte begleitet. Part 1*. Arnz & Co., Düsseldorf, 32 pp.
- Sterling, E.J., Hurley, M.M. & Le, M.D. (2006) *Vietnam: a natural history*. Yale University Press, New Haven and London, 423 pp.
- Stuart, B.L. & Heatwole, H.F. (2004) A new *Philautus* (Amphibia: Rhacophoridae) from northern Laos. *Asiatic Herpetological Research*, 10, 17–21.
- Stuart, B.L., Inger, R.F. & Voris, H.K. (2006) High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian frogs. *Biology Letters*, 2, 470–474.
<http://dx.doi.org/10.1098/rsbl.2006.0505>
- Stuart, B.L., Rowley, J.J.L., Neang, T., Emmett, D.A. & Som, S. (2010) Significant new records of amphibians and reptiles from Virachey National Park, northeastern Cambodia. *Cambodian Journal of Natural History*, 2010, 38–47.
- Stuart, B.L., Rowley, J.J.L., Tran, D.T.A., Le, D.T.T. & Hoang, H.D. (2011) The *Leptobrachium* (Anura: Megophryidae) of the Langbian Plateau, southern Vietnam, with description of a new species. *Zootaxa*, 2804, 25–40.
- Stuebing, R.B. & Wong, A. (2000) A new species of frog, *Philautus erythrophthalmus* (Rhacophoridae) from southwestern Sabah, Malaysia. *The Raffles Bulletin of Zoology*, 48, 293–296.
- Swofford, D.L. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony *(and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Tschudi, J.J.v. (1838) *Classification der Batrachier, mit Berücksichtigung der fossilen Thiere dieser Abtheilung der Reptilien*. Petitpierre, Neuchâtel, 99 pp.
- Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. & Vences, M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences*, 106, 8267–8272.
<http://dx.doi.org/10.1073/pnas.0810821106>
- Yu, G., Rao, D., Yang, J. & Zhang, M. (2008) Phylogenetic relationships among Rhacophorinae (Rhacophoridae, Anura, Amphibia), with an emphasis on the Chinese species. *Zoological Journal of the Linnean Society*, 153, 733–749.
<http://dx.doi.org/10.1111/j.1096-3642.2008.00404.x>